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RESEARCH PAPER

Beneficial effects of cold-moist stratification on seed germination behaviors of *Abies pindrow* and *Picea smithiana*

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Abstract: A study was conducted to evaluate the effect of GA_3, moist–chilling and temperature on seed germination of *Abies pindrow* and *Picea smithiana* from five different provenances. Seeds were soaked in GA_3 (10 mg·L^{-1}) for 24 h, then chilled at 3–5°C for 15 days. Four temperature regimes viz. 10°C, 15°C, 20°C and 25°C were used for stimulating seed germination. Results showed that soaking and chilling significantly increased germination percentage. The germination percentage was highest at 10°C. Overall results showed that soaking seeds in GA_3 (10 mg·L^{-1}) for 24 h, moist chilling for 15 days, and germinating at 10°C produced an effective germination in both the species studied. The statistical analysis of the data proclaimed significant effect of treatment, temperature, provenance and treatment with temperature interactions on seed germination.

Keywords: stratification; provenance; seed germination; GA_3; silver fir; spruce

Introduction

The Western Himalayan temperate Forests are distinguished as Moru Oak Forests, Moist Deodar Forests, Western Mixed Coniferous Forests, Low Level Blue pine forests, Kharsu Oak Forests and West Himalayan Upper Oak and fir forests (Champion et al. 1968). The most common coniferous species in these forests are Blue Pine (*Pinus wallichiana* A. B. Jacks), Himalayan cedar (*Cedrus deodara* Royal ex D. Don), Himalayan cypress (*Cupressus torulosa* Don), Spruce (*Picea smithiana* wall. Boiss), Silver fir (*Abies pindrow* spach.) and Himalayan Yew (*Taxus baccata* Linn.). The natural regeneration of silver fir, spruce and Himalayan yew is generally poor and first attention to this problem was paid by Redcliffe (1906). Since then, the problem of natural regeneration of these species has been constantly engaging the attention of the forest scientists. A number of factors are considered responsible for the absence of natural regeneration of these species, such as lack of adequate light on the forest floor, dense weed growth (Troup 1921), thick layer of humus (Troup 1921; Taylor et.al. 1934; Glover 1936; Kaul 1970), accumulation of debris (Hafizullah 1970), and continuous grazing (Redcliffe 1906; Flewett 1930; Sufi 1970). Infrequent seed years and low germinative capacity of the seeds could be also considered being important factors contributing poor natural regeneration.

The reproductive system of conifers is exclusively sexual and the natural regeneration, in turn, depends on the production, dispersal and germination capacity of seeds and successful establishment of seedlings. Seed germination of most temperate coniferous species is inhibited by evolved trait seed dormancy (Leadem 1986; West et al. 1970; Singh 1989). Cold-moist stratification is a commonly used practice to break dormancy in seeds and to attain vigorous, speedy, maximum, and uniform germination for laboratory testing, green house and nursery sowing (Fowler et al. 1964), which has been reported in many studies on conifers (Mergen 1963; Fowler et al. 1964; Roberts et al. 1982; Wang et al. 2000;) and several hardwood seeds (Schopmeyer 1974; Villiers 1971; Nikolaeva 1977; Bevington 1986; Barnett et al. 1978; Farmer et al. 1972; Farmer 1974; Farmer et al. 1981).

The plant hormone, GA_3, plays an important role in control of the various physiological processes in plant growth and development including seed germination, shoot growth, cell division, internode elongation and the formation of flower buds. Gibberellin is well known to break dormancy of seeds and buds in many plants (Brian et al. 1959; Stuart et al. 1961; Weaver 1959). The Gibberellic acid (GA_3) has been reported to promote germination of seeds (Vogt 1970; Krishnamurthy 1973; Chandra et al. 1976). However, the germination percentage increased in the seeds of *Nothofagus obliqua*, when they were pre-chilled after soaking in GA_3 solutions for 24 hours (Shafiq 1980). Moist chilling and gibberellin treatments have been reported very effective for seed germination in some woody species, viz. *Aesculus hippocastanum* (Tompsett et al. 1998), *Pinus taeda* (Wu et al. 2001), *Ginkgo biloba* (Wilson et al. 2004), *Acer pensylvani-*

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cum (Bourgoin et al. 2004), *Aribotrya japonica* (El-Dengawy, 2005), *Larix deciduas* (Gorian et al. 2007) and *Prunus campanulata* (Chen et al. 2007). Variation in germination can be manifested through provenance tests designed to assess the degree and pattern of variation across species ranges. Such tests are actually based on the phenotypic variations among seed lots from provenances, and not on genetic variation. Rowe (1964), and Baskin and Baskin (1973) have noted that the difference in seed characteristics of ecologically important provenances may also be due to genetic variability. Considering all the aforesaid facts, the present study aims at understanding the effect of pre-chilling, after soaking in GA_3 solutions for 24 hours, on the seed germination of different provenances of *Abies pindrow,* and *Picea smithiana.*

Materials and methods

Selected seed provenances

The seeds of *A. pindrow* and *P. smithiana* were collected from 5 different provenances from Garhwal Himalaya, India. The details of the provenances selected within the study area were presented in Table 1 and Fig. 1.

Table 1. Geographical and meteorological description of the selected provenances of *A. pindrow* and *P. smithiana*

Provenances	Species occurred	Altitude (m)	Latitude (N)	Longitude (E)	Temperature (°C)		Mean annual rainfall (mm)
					Min.	Max.	
Bharsar	*A. pindrow*	2697	30º24'	79º31'	-0.70	27.5	1084.00
Dudhatoli	*A. pindrow*	3122	30º5'	79º12'	-0.65	25.8	1935.00
Ransolikhal	*A. pindrow*	2750	30º25'	79º17'	-0.56	26.9	1475.00
Surkanda	*A. pindrow*	3030	30º27'	79º18'	-0.75	25.3	1595.00
Tapovan	*A. pindrow & P. smithiana*	3798	30º31'	79º36'	-0.84	24.5	1892.00
Banjbagad	*P. smithiana*	2775	30º15'	79º34'	1.13	30.8	1276.00
Hanumanchatti	*P. smithiana*	2880	30º41'	79º30'	-0.10	25.3	2098.00
Helang	*P. smithiana*	2595	30º33'	79º37'	1.34	28.9	1860.00
Pandukeshwar	*P. smithiana*	2657	30º31'	79º32'	-0.91	27.0	1932.00

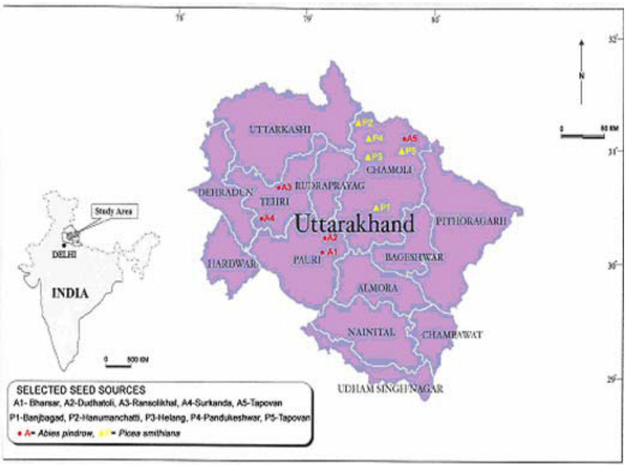

Fig. 1 Location map of the study area

The seed germination tests were conducted under laboratory conditions at various constant temperatures *viz*., 10°C, 15 °C, 20°C and 25°C inside a seed germinator (Model No. 8LT-SGL CALTAN). The seeds of all the provenances of both species were germinated at all the aforesaid temperatures to obtain the best temperature range for seed germination after applying following treatments: Treatment 1 (soaking of the seeds in distilled water at room temperature (25°C) for 24 hours) was as control treatment. Treatment 2 (seeds in Gibberellic acid (GA_3 10 $mg \cdot L^{-1}$) were soaked at room temperature for 24 hour and then chilled for 15 days (at 3−5°C).

For germination, five replicates of 20 pre-treated seeds were placed in Petri dishes containing two filter papers, kept in the germinator, and maintained at desired temperatures in light condition. Observation data were recorded daily upto 21 days. Radical emergence was taken as the criteria for germinability. The recorded data on seed germination were quantified in terms of germination percentage and germination value. The germination percentage was the value of seeds germinated at the completion of the germination period, whereas, germination value is an index, combining speed and completeness of germination. The germination value according to Czabator (1962) can be expressed as:

$$G_V = P_V \times M_{DG} \quad (1)$$

where, G_V is the germination value, P_V the peak value of germination, and M_{DG} is the mean daily germination.

Statistical analysis

The statistical analysis was conducted on mean values and the analysis of variance (ANOVA) was performed using SPSS package. The critical difference (C_d) was calculated as:

$$C_d = S_{Ed} \times t_{0.01} \quad (2)$$

where, S_{Ed} is the standard error of difference calculated as

$$S_{Ed} = \sqrt{2}\mathrm{Me} / r \quad (3)$$

where, Me is the mean sum of square due to error, and r is the number of replicates.

Results and discussion

Germination of seeds of various provenances of *A. pindrow* and *P. smithiana* after pre-soaking and pre-chilling treatments under different temperature regimes, (10°C, 15°C, 20°C and 25 °C) has

yielded significant differences in seed germination. The detailed treatment-temperature interactions are as following. Five different provenances, viz. Bharsar, Dudhatoli, Ransolikhal, Surkanda and Tapovan of *Abies pindrow* manifested maximum germination percentage at 10 °C, for both control and GA_3 treated chilled seeds. The seeds of different provenances which had given distilled water treatment proclaimed a germination percentage range of 21%–32 % at 10°C, 14%–18% at 15°C, 15%–22% at 20°C and 10%–19% at 25°C, whereas, pre-chilled seeds manifested 34%–56%, 29%–47%, 28%–33% and 25%–30% germination at 10°C, 15°C, 20°C, and 25°C, respectively (Table 2). Similarly, the seed germination of five provenances, i.e, Banjbagad, Hanumanchatti, Helang, Pandukeshwar and Tapovan, of *P. smithiana* also revealed the maximum germination percentage at 10°C. The range of germination percentage at 10°C, 15°C, 20°C and 25°C for both control and prechilled seeds was from 31%–37% and 52%–72%, respectively; at 10°C, from 22%–30% and 32%–55%, respectively; at 15 °C, from 25%–30% and 39%–48%, respectively; at 20°C and 15%–26% and 32%–47%, respectively at 25°C (Table 3).

Table 2. Effect of different treatments and temperatures on seed germination percentage and germination value in different provenances of *Abies pindrow* (Italics represent germination value)

Treatments	Provenances				
	Bharsar	Dudhatoli	Ransolikhal	Surkanda	Tapovan
			10°C		
Control	22±3.75	26±4.86	26±4.01	21±2.92	32±2.00
	0.34±0.08	*0.26±0.05*	*0.54±0.18*	*0.41±0.08*	*0.67±0.15*
Chilling	34±1.87	56±5.80	45±4.19	45±5.49	52±5.84
	0.53±0.10	*0.80±0.21*	*0.63±0.11*	*0.58±0.18*	*0.56±0.07*
GA_3 (10 mg·L^{-1})	28±2.55	41±4.86	45±4.19	33±3.40	34±4.86
	0.39±0.04	*0.64±0.14*	*0.69±0.12*	*0.48±0.11*	*0.47±0.14*
			15°C		
Control	14±4.01	17±2.55	17±1.22	17±2.55	18±4.37
	0.30±0.10	*0.25±0.07*	*0.22±0.06*	*0.39±0.09*	*0.25±0.14*
Chilling	29±1.87	43±3.40	34±3.68	37±2.00	47±5.39
	0.29±0.02	*0.54±0.09*	*0.51±0.11*	*0.45±0.09*	*0.64±0.11*
GA_3 (10 mg·L^{-1})	27±5.16	25±1.58	21±2.92	21±4.31	31±2.92
	0.47±0.15	*0.35±0.03*	*0.38±0.13*	*0.29±0.07*	*0.44±0.05*
			20°C		
Control	15±3.17	15±3.54	22±9.59	21±7.50	17±2.00
	0.15±0.04	*0.29±0.11*	*0.49±0.38*	*0.12±0.02*	*0.11±0.01*
Chilling	31±4.90	33±2.55	28±3.00	33±4.64	30±2.74
	0.26±0.06	*0.39±0.08*	*0.41±0.07*	*0.30±0.06*	*0.33±0.03*
GA_3 (10 mg·L^{-1})	23±6.26	28±3.40	25±2.74	18±4.07	25±1.58
	0.44±0.08	*0.47±0.17*	*0.27±0.07*	*0.20±0.06*	*0.26±0.03*
			25°C		
Control	10±8.28	12±2.00	19±1.00	10±3.17	10±2.74
	0.30±0.28	*0.19±0.05*	*0.26±0.05*	*0.09±0.03*	*0.10±0.05*
Chilling	27±3.00	30±5.71	27±4.64	30±6.53	25±3.17
	0.36±0.05	*0.43±0.13*	*0.43±0.13*	*0.39±0.11*	*0.27±0.05*
GA_3 (10 mg·L^{-1})	20±6.53	16±4.31	23±2.00	26±4.86	21±2.45
	0.20±0.09	*0.16±0.05*	*0.29±0.05*	*0.46±0.17*	*0.28±0.05*

A critical review of the data presented in Tables 2 & 3 reveals that among all the selected temperature regimes, 10°C was the best temperature for the seed germination of both species, as the highest germination percentage and germination value was observed in this constant temperature, whereas, the least percentage of germination and germination value was recorded at 25°C. However, the seeds which were followed chilling treatment after soaking in GA_3 for 24 h manifested highest percentage of germination in all the selected provenances of both species. On the other hand, seeds treated with distilled water (as control) exhibited poor germination percentage and germination value in all the provenances of *A. pindrow*, and *P. smithiana*. In nature, dormant seeds of most temperate conifer's species are exposed to cold wet conditions during winter, which germinate when temperature rise in early spring. The dormant seeds of such species would germinate at controlled, low temperature in the laboratory (Edwards 2004). Among different provenances of both species, the Dudhatoli provenance of *A. pindrow* and Tabovan provenence of *P. smithiana* were the most successful in respect of germination percentage and germination value. The statistical analysis of the data revealed significant effect of treatment, temperature, provenance and treatment with temperature interactions on seed germination in both the studied taxa (Table 4).

Moist chilling has long been recognized as a useful method of treating seeds to improve the rate and percentage of germinability (Outcall 1991) in addition to other pre-sowing treatments that increase germination (Heydecker et al. 1977). The treatment may also facilitate germination at sub-optimal temperatures (10–20°C), which is particularly important for spring sowing in nurseries in temperate climates. Allen (1960) was of the opinion that the longer the chilling period, the better was the germination of the coniferous seeds. Improved germination by stratification at

different time period has been reported in *Ginkgo biloba* for 12 weeks stratification period (West et al. 1970), *Ceanothus sanguinus* for 4 months at 2–5 °C (Radwan et al. 1977), *Carpinus caroliana* for 18 weeks at 4–5 °C (Bretzloff et al. 1979), *Cedrus deodara* for one week (Thapliyal et al. 1980) and *Picea smithiana* for two months at 2°C (Singh 1989). In the present study, moist chilling after GA_3 treatment has resulted in better germination for seeds, which was duly supported by many other studies (Roos et al. 1971; Willemsen et al. 1972). Nevertheless, the stratification-redry method has been shown to improve germination in Pacific silver fir *(Abies amabilis*), (Edwards 1982; 1997; Leadem 1986), subalpine fir *(A. lasiocarpa*), (Leadem 1988, 1989), and Nordmann fir *(A. nordmanniana*), (Jensen 1997). However, controlling fir seed moisture content during stratification is not a new idea, having been recommended for prolonged pretreatment of seeds of hybrid firs (Wright 1950). Additionally, reduced seed moisture content was reported for pretreatment of Guatemalan fir (*A. guatemalensis*), (Donahue et al. 1985).

Table 3. Effect of different treatment and temperature on germination percentage and germination value in different provenances of *Picea smithiana* (Italics represent germination value)

Treatments	Provenances				
	Banjbagad	Hanumanchatti	Helang	Pandukeshwar	Tapovan
			10°C		
Control	31±1.86	31±6.61	37±2.25	36±3.68	27±3.75
	0.54±0.11	*0.83±0.29*	*0.69±0.09*	*1.26±0.30*	*0.68±0.09*
Chilling	52±3.40	63±6.05	54±8.59	59±5.35	72±7.53
	0.73±0.18	*1.17±0.21*	*0.82±0.27*	*1.48±0.29*	1.26±0.31
GA_3 (10 mg·L^{-1})	42±5.62	53±4.07	45±5.01	52±3.75	56±6.01
	0.83±0.29	*0.93±0.18*	*1.05±0.24*	*1.07±0.25*	*1.29±0.07*
			15°C		
Control	29±2.92	22±2.55	29±1.12	30±1.58	22±2.00
	0.49±0.09	*0.41±0.10*	*0.59±0.05*	*0.61±0.05*	*0.82±0.17*
Chilling	32±5.16	41±5.80	35±5.25	50±3.17	55±4.19
	0.31±0.04	*0.50±0.09*	*0.38±0.10*	*0.99±0.32*	*0.84±0.13*
GA_3 (10 mg·L^{-1})	38±3.00	30±4.48	38±6.65	38±2.00	36±5.58
	1.07±0.22	*0.64±0.13*	*0.59±0.12*	*0.78±0.13*	*0.61±0.05*
			20°C		
Control	29±6.22	26±4.31	26±4.31	25±1.58	30±2.74
	0.57±0.13	*0.55±0.09*	*0.46±0.08*	*0.42±0.06*	*0.71±0.21*
Chilling	39±4.31	48±6.05	48±6.05	45±4.75	45±5.25
	0.49±0.07	*0.91±0.18*	*0.62±0.16*	*0.41±0.15*	*0.57±0.11*
GA_3 (10 mg·L^{-1})	36±3.68	43±3.75	43±3.75	54±11.90	42±2.00
	0.85±0.18	*1.02±0.13*	*1.16±0.39*	*1.57±0.53*	*1.22±0.13*
			25°C		
Control	26±4.01	21±1.87	21±1.87	25±3.54	15±5.71
	0.42±0.10	*0.35±0.15*	*0.30±0.09*	*0.41±0.15*	*0.22±0.10*
Chilling	32±3.00	42±5.84	42±5.84	57±5.16	31±3.32
	0.30±0.03	*0.49±0.12*	*0.48±0.10*	*1.08±0.34*	*0.36±0.06*
GA_3 (10 mg·L^{-1})	30±2.74	28±4.37	28±4.37	35±4.48	24±2.45
	0.41±0.07	*0.70±0.24*	*0.45±0.12*	*0.84±0.23*	*0.46±0.11*

Table 4. ANOVA effects of provenance, temperature, treatment and treatment with **temperature on seed germination of *Abies pindrow* and *Picea smithiana***

Source of variation	*Abies pindrow*							
	d. f	SS	MSS	F-ratio	F-critical		CD	
					5%	1%	5%	1%
Provenances	4	0.79	0.1975	5.64**	3.26	5.41	0.26	0.36
Temperature	3	1.89	0.63	18**	3.49	5.95	0.28	0.40
Error[a]	12	0.42	0.035					
Treatment	3	1.56	0.52	4.7**	2.79	4.26	0.47	0.63
Treatment X Temperature	9	2.16	0.24	2.16*	2.08	2.81	0.29	0.39
Split plot Error[b]	48	5.31	0.1106					

Source of variation	*Picea smithiana*							
	d. f	SS	MSS	F-ratio	F-critical		CD	
					5%	1%	5%	1%
Provenances	4	1.46	0.365	6.08**	3.26	5.41	0.34	0.47
Temperature	3	3.14	1.04667	17.43**	3.49	5.95	0.37	0.53
Error[a]	12	0.72	0.06					
Treatment	3	1.46	0.48667	1.87	2.79	4.26	0.72	0.95
Treatment X Temperature	9	7.45	0.82778	3.19**	2.08	2.81	0.46	0.61
Split plot Error[b]	48	12.45	0.25938					

Notes: *----Significant at 5% and **----significant at 1%; MSS---- mean sum of squares SS---- Sum of squares.

For shallow-dormant seeds of Douglas-fir (*Pseudotsuga menziesii*), lodgepole pine (*Pinus contorta*) and Stika spruce (*Picea sitchensis*), moist chilling is not only a requirement to alleviate dormancy, but a prolonged period of chilling is necessary to achieve rapid and uniform germination under the low temperature in early spring (Jones et al. 1994; Jinks et al. 1996). Moist chilling for 15 days did improve the rate and percentage of germination of *A. pindrow* and *P. smithiana* seeds at 10–15°C over 21 days. This effect of moist-chilling on the activation of germination may be utilized for short-term maintenance of seeds in a moistened condition at 3–5°C, which could be considered important for forestry practices, and afforestation programmes in temperate regions. Further, the results of the present study suggest that the seeds of *A. pindrow* and *P. smithiana* are dormant. The level of dormancy may vary from one provenance to another even for seeds from the same parent trees, among parent trees in the same stand in any one crop, among cones on the same parent tree, and from seed to seed in the same cone. Thus, in any seed lot, some seeds may be non-dormant, slightly dormant, somewhat dormant, while others are very dormant. Standard stratification/prechilling treatments to promote germination could accommodate such wide variations.

Exogenous application of GA_3 has been reported to be effective in breaking dormancy and in substituting for a cold stratification requirement in many seeds (Chien et al. 2002; Hidayati et al. 2000; Chen et al. 2005). Results of our study showed that the application of soaking seeds in 10-mg·L^{-1} GA_3 solution for 24 h followed by 2-week moist chilling at 3–5°C was very effective in enhancing germination, however, soaking seeds in GA_3 (10mg·L^{-1}) for 24 h produced average germination for all five seed sources of both species studied. Gibberellic Acid-3 (GA_3) is a naturally occurring plant growth regulator which may cause a variety of effects including the stimulation of seed germination in some cases. GA_3 occurs naturally in the seeds of many species. Studies on woody plants indicated that the GA_3 content of seeds increases during cold stratification. For example, cold stratification induced an increase in GA_3 and in GA_7 in peach (*P. persica*) seeds (Mathur et al. 1971), in GA_1 in *Corylus avellana* embryos (Williams et al. 1974), in GA4b in apple seeds (Halin´ ska et al. 1987) and in GA_1, GA_3 and GA_4 in *P. buergeriana* seeds (Chen et al. 2005). Cold stratification induced an increase of GA_3 levels in embryos of European hazel (*Corylus avellana*), suggesting that gibberellins synthesized during cold treatment were responsible for dormancy break (Williams et al. 1974). Yamauchi et al. (2004) demonstrated that a gene involved in GA_3 biosynthesis in seeds of *Arabidopsis thaliana* was activated by cold stratification at 48°C. Increase in tissue sensitivity to gibberellins during cold stratification is another factor that may be involved in controlling seed germination (Derkx et al. 1993; Koornneef et al. 2002).

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